

Vicia faba Fungal diseases: Could nanostructures drug delivery systems be a novel paradigm for therapy?

Mohammed N. A. Hasaneen

mohammedgib@mans.edu.eg

Mansoura University

Sara H. Helal

Mansoura University

Mustafa M. El-Zayat

New Mansoura University

Heba M. M. Abdel-Aziz

Mansoura University

Research Article

Keywords: biosafety, carbon nanotubes, chitosan nanoparticles, chocolate spot, fluconazole, growth and yield parameters, leaf spot, nano-drug delivery, nystatin, solid lipid nanoparticles, TEM-Vicia faba, uptake and translocation

Posted Date: March 21st, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4125766/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

New drug delivery strategies have become a pioneer and novel approach in controlling plant diseases in application research nowadays. In this paper, we investigate the delivery of chitosan nanoparticles (CSNPs), carbon nanotubes (CNTs) and solid lipid nanoparticles (SLNPs) loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics for diseased *Vicia faba* plants by foliar uptake. CSNPs-NYS or FLZ, CNTs-NYS or FLZ and SLNPs-NYS or FLZ were easily applied to leaf surfaces and enter the stomata via gas uptake, avoiding direct interaction with soil systems. The uptake, translocation and biosafety of nano-drug delivery systems inside *Vicia faba* plants was investigated by transmission electron microscopy. The results obtained revealed that all different nanoparticles were taken up and transported through phloem tissues, in particular, sieve tubes of either healthy, infected or recovered *Vicia faba* plants. On the other hand, growth and yield developmental parameters in the present study including shoot length, number of pods per plants, weight of pod, number of seeds per pod, weight of seed, seed yield per plant, harvest index, crop index and mobilization index of healthy and recovered *Vicia faba* plants showed, in general, significant increases. The net results indicate the possibility of these formulation to protect plant from the disease with a sequence of: solid lipid nanoparticles-FLZ; 346 %> CSNPs+FLZ; 315 %> CNTs+FLZ; 274%

INTRODUCTION

Every year, agricultural production decreases due to plant disease. Among different natural and artificial protection methods, the most widespread is using pesticides [1]. Recently, there have been lots of discussions regarding environmental hazards due to excessive use of pesticides. Thus, scientists active in the field of agricultural are trying to find other measures to battle pests [2]. Thus, we urgently need biological control agents that can get rid of pathogens without adversely affecting the environment [3]. Recent developments in nanoscale engineering have presented a new class of particulate bio nanotechnology that utilizes biomimicry to better integrate antigen and adjuvant [4].

Nanostructured systems could be excellent carriers for antifungal drugs, reducing toxicity and targeting their action. The use of nanostructured systems for antifungal therapy began in the 1990s, with the appearance of lipid formulations. Different antifungal drug delivery systems, such as liposomes, carriers based on solid lipids and nanostructure lipids, polymeric nanoparticles, and others had been reported. All these delivery systems have advantages and disadvantages. Main advantages are the improvement in the antifungal properties, such as bioavailability, reduction in toxicity, and target tissue, which facilitates innovative therapeutic techniques [5]. Faba bean is one of the most important economic winter crops in Egypt and belonging to family Fabaceae, which is native to North and Southwest of Africa [6, 7].

Faba bean plants are attacked by the fungal pathogens such as chocolate spot (*Botrytis fabae*) and Alternaria leaf spot (*Alternaria alternata*) which are responsible to cause considerable losses in the yield and its component [6]. In recent decades, bacteria and fungi have developed considerable resistance to many traditional and modern synthetic drugs [8]. In this context, nanoparticles (NPs) can also overcome the drug resistance mechanisms, related to decreased absorption, increased drug efflux from microbial cells, biofilm formation, or intracellularly [9]. Finally, NPs deliver the highest dose of antimicrobial agents specifically to the site of infection, thus overcoming drug resistance with less adverse effects on the patient [10].

The present study was carried out to evaluate the efficacy of chitosan nanoparticles, carbon nanotubes and solid lipid nanoparticles either singly or loaded with nystatin and fluconazole antifungal antibiotics, as nano-drug delivery systems, as novel techniques for controlling *Botrytis fabae* and *Alternaria alternata* fungal pathogens. *In vivo* studies deal with the effect of tested nano-drug delivery strategies on growth and productivity of *Vicia faba* plant, uptake and translocation and biosafety of nanomaterials used.

MATERIALS AND METHODS

Inoculum and inoculations

The spore suspension was inoculated at different places on the appear leaflet samples. The leaflets were kept moist. Each concentration contained 4 replicates. The development of infection was assessed by measuring the expansion of the lesions from third to sixth day after incubation.

Effects of tested nanoparticles on infection with *Botrytis fabae* and *Alternaria alternata* under field conditions.

The efficacy of nanoparticles either singly or loaded with antifungal antibiotics against *B.fabae* and *A.alternata* were detected at field conditions. Faba bean seeds of Sakha 1 Cv. (susceptible cultivar to leaf spot disease) used in these field experiments were obtained from the Agricultural Research Center, Ministry of Agriculture, Mansoura, Egypt, and were growing in field rows. Our work complies with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. Based on the obtained data from the previous field and laboratory experiments [11], optimum effective concentrations of nanomaterials and/ or minimum inhibitory concentration (MIC) of either nystatin or fluconazole were applied on the leaves of plants for 3 weeks before and after the disease outbreak. Growth and developmental parameters of *Vicia faba* yield were determined.

Uptake and translocation studies

The uptake and translocation of nanoparticles were determined inside *Vicia faba* tissues by examination with transmission electron microscopy (TEM).

Biosafety measurements

The biosafety of yielded *Vicia faba* seeds was determined by examination of the yielded seeds under transmission electron microscope (TEM) [11].

Statistical Analysis

The obtained data were analyzed by one-way analysis of variance (ANOVA), the mean values were compared using the Post Hoc L.S.D. (least significant difference) at 0.05 probability level.

Results

Characterization of Chocolate spot disease and leaf spot disease and recovery

Careful examination of figures 1-2 revealed that symptoms of chocolate spot disease appeared on leaves, stems flowers and pod tissues. Infection started as small reddish-brown circular spots on leaves, stems and flowers. The spots on leaves and stems enlarged and developed a grey, dead center with a red-brown margin. In the present study, two stages of the disease have been observed. The non-aggressive stage known by the small circular and discrete reddish spots with darker margins visible on one side of leaf; and the aggressive stage where the small spots merge and coalesce to form irregular larger dark-brown lesions involving the entire leaf surface. Under favorable conditions, the disease spread quickly causing severe defoliation, flowers drop, stem collapse, tissue necrosis and finally plant death (figures 1-2).

Careful examination of figures 3-4 showed that initially lesions were brown, water soaked, circular to irregular shape on the stem, pod, and other parts of the plant. These dark brown leaf spots often had a zoned pattern of concentric brown rings with dark margins which gave the spots a target-like appearance. The fungus showed that older leaves were usually attacked first but the disease progressed upward and affected leaves which turned yellowish, became senescent and either dried up and dropped or fell off. In the later stage of the disease, the leaves became blighted from the margin to the center and most of the diseased plants defoliated completely.

Uptake and translocation of nanomaterials

Transmission Electron Microscopy (TEM) for investigation of plant leaves (46-day-old) after applying chitosan nanoparticles, carbon nanotubes and solid lipid nanoparticles either singly or loaded with nystatin and fluconazole antifungal antibiotics was carried out in the present study to indicate the presence or absence of nanomaterials as a result of taken up through leaves of broad bean plants at late vegetative growth and developmental stage.

Careful examination of figures 1-6 derived from TEM images of broad bean leaves (46-day-old) revealed the following main points:

- Complete absence of nanomaterials either singly or loaded with nystatin and fluconazole inside epidermal cells, cortical cells, spongy tissues, or palisade tissues but they were existed in vascular system of broad bean leaves treated with chitosan nanoparticles, carbon nanotubes and solid lipid nanoparticles either singly or loaded with nystatin and fluconazole antifungal antibiotics (figures 4-9).
- Figures 4-9 showed that foliar application of the three nanoparticles either singly or in combination with nystatin and fluconazole to broad bean plants and confirm that the uptake and translocation of nanoparticles were achieved by phloem tissues, in particular, sieve tubes cells.
- It is worth to mention that the observed results concerning the uptake and translocation of nanomaterials by broad bean plants through the entire period of the experiment might lead to conclude that the main pathway for uptake and translocation of nanomaterials either singly or loaded with nystatin and fluconazole was via the cells of the phloem sieve tubes.

Changes in growth, yield and developmental parameters

Foliar application of either CSNPs, CNTs or SLNPs singly or loaded with nystatin and fluconazole antibiotics led to significant variable increases in all yield growth and developmental parameters determined as compared with control diseased values. The magnitude of increase was, in general, most pronounced with solid lipid nanoparticles loaded with nystatin and fluconazole antifungal antibiotics (tables 1 and 2).

Furthermore, harvest index, crop index and mobilization index values of broad bean plants variously treated with nanomaterials either singly or loaded with nystatin and fluconazole antifungal antibiotics were found to be variously changed corresponding to treatment type, way and levels of treatments as compared to values of diseased plants. Thus, in general, as evident from tables 1 and 2 and as compared to diseased plants, the calculated percentage ratio of increase in harvest index was as follows: strategy C > strategy A > strategy B was displayed for *Botrytis fabae* with respect to treatment of infected broad bean plants with nano-drug delivery strategies. In relation to control diseased broad bean plants, the calculated percentage improvement in crop index of the yielded broad bean plants was arranged as follows: SLNPs + FLZ > SLNPs + NYS > CSNPs + FLZ > CSNPs + NYS > CNTs + FLZ > CNTs + NYS > diseased plants. Furthermore, mobilization index of the yielded broad bean crop as compared with control diseased plants was as follows: SLNPs + FLZ > SLNPs + NYS > CSNPs + FLZ > CSNPs + NYS > CNTs + FLZ > CNTs + NYS > diseased plants.

As compared with control diseased plants, it is worth to mention that the growth and developmental yield parameters of broad bean plants variously treated with nanomaterials either singly or loaded with nystatin and fluconazole antifungal antibiotics showed significant improvement percentage of shoot length, number of pods per plant, pod weight per plant, number of seeds per plant, crop yield per plant and seed yield per plant. The magnitude of increased improvement in these growth and developmental yield parameters was most pronounced with solid lipid nanoparticles loaded with nystatin or fluconazole as nano-drug delivery system (tables 1 and 2).

Table 1: Effect of foliar application of different nanomaterials treatments either singly or loaded with nystatin and fluconazole on yield developmental parameters of Faba bean plants grown in clay-sandy soil (before and after infection with *Botrytis fabae*). In each case, the % change from the control and the % recovery from the infected plant are also shown. Each value is a mean of five replicates. *The values listed are significantly different from control at $p \geq 0.05$.

Parameter Treatment	Plant height (cm plant ⁻¹)	% change	% Recov	Shoot length (cm plant ⁻¹)	% change	% Recov	No. of pods/ plant	% change	% Recov	Pod weight (g pod ⁻¹)	% change	% Recov
Control	61.11	—	—	54.38	—	—	4.00	—	—	1.54	—	—
Healthy Plant												
CNTs	60.32*	-1.29	—	53.21*	-2.15	—	4.00	0.00	—	1.49*	-3.25	—
CSNPs	66.71*	9.16	—	61.31*	12.74	—	5.00*	25.00	—	2.38*	54.55	—
SLNPs	64.12*	4.93	—	59.00*	8.50	—	4.00	0.00	—	1.77*	14.94	—
Infected Plant with <i>Botrytis fabae</i>												
Infected plant	51.33*	-16.00	—	42.67*	-21.53	—	3.00*	-25.00	—	0.99*	-35.71	—
NYS	63.65*	4.16	24.00	55.42*	1.91	29.88	4.00	0.00	33.33	2.06*	33.77	108.08
FLZ	67.73*	10.83	31.95	60.91*	12.01	42.75	4.00	0.00	33.33	2.19*	42.21	121.21
Strategy A												
A1: CSNPs	54.70*	-10.49	6.57	49.31*	-9.32	15.56	3.00*	-25.00	0.00	1.26*	-18.18	27.27
A2: CSNPs-NYS	65.73*	7.56	28.05	59.16*	8.79	38.65	5.00*	25.00	66.67	2.25*	46.10	127.27
A3: CSNPs-FLZ	64.72*	5.91	26.09	58.30*	7.21	36.63	5.00*	25.00	66.67	2.21*	43.51	123.23
Strategy B												
B1: CNTs	51.78*	-15.27	0.88	41.94*	-22.88	-1.71	3.00*	-25.00	0.00	1.05*	-31.82	6.06
B2: CNTs-NYS	60.41*	-1.15	17.69	53.70*	-1.25	25.85	4.00	0.00	33.33	1.82*	18.18	83.84
B3: CNTs-FLZ	61.88*	1.26	20.55	51.78*	-4.78	21.35	4.00	0.00	33.33	1.71*	11.04	72.73
Strategy C												
C1: SLNPs	54.55*	-10.73	6.27	46.17*	-15.10	8.20	3.00*	-25.00	0.00	1.16*	-24.68	17.17
C2: SLNPs-NYS	70.83*	15.91	37.99	64.49*	18.59	51.14	5.00*	25.00	66.67	2.28*	48.05	130.30
C3: SLNPs-FLZ	68.59*	12.24	33.63	60.25*	10.79	41.20	5.00*	25.00	66.67	2.50*	62.34	152.53

Cont. table 1:

Parameter	No. of seeds/ pod	% change	% Recov	Seed weight (g seed ⁻¹)	% change	% Recov	Crop yield /plant (g plant ⁻¹)	% change	% Recov	Seed yield /plant (g plant ⁻¹)	% change	% Recov	Straw yield /plant (g plant ⁻¹)	% change
Treatment														
Control	3.00	—	—	0.43	—	—	6.59	—	—	5.20	—	—	8.84	—
Healthy Plant														
CNTs	3.00	0.00	—	0.35	-18.60	—	6.37*	-3.34	—	4.07*	-21.73	—	8.14*	-7.92
CSNPs	3.00	0.00	—	0.60*	39.53	—	11.30*	71.47	—	8.97*	72.50	—	8.33*	-5.77
SLNPs	3.00	0.00	—	0.45	4.65	—	7.49*	13.66	—	5.48*	5.38	—	7.04*	-20.36
Infected Plant with <i>Botrytis fabae</i>														
Infected plant	2.00*	-33.33	—	0.23*	-46.51	—	2.96*	-55.08	—	1.35*	-74.04	—	8.86	0.23
NYS	3.00	0.00	50.00	0.45	4.65	95.65	8.03*	21.85	171.28	5.99*	15.19	343.70	8.52*	-3.62
FLZ	3.00	0.00	50.00	0.53*	23.26	130.43	9.00*	36.57	204.05	6.34*	21.92	369.63	8.40*	-4.98
Strategy A														
A1: CSNPs	2.00*	-33.33	0.00	0.38	-11.63	65.22	4.73*	-28.22	59.80	2.44*	-53.08	80.74	7.82*	-11.54
A2: CSNPs-NYS	3.00	0.00	50.00	0.57*	32.56	147.83	10.93*	65.86	269.26	8.79*	69.04	551.11	7.56*	-14.48
A3: CSNPs-FLZ	3.00	0.00	50.00	0.56*	30.23	143.48	10.85*	64.64	266.55	8.62*	65.77	538.52	7.08*	-19.91
Strategy B														
B1: CNTs	2.00*	-33.33	0.00	0.31*	-27.91	34.78	3.27*	-50.38	10.47	1.83*	-64.81	35.56	9.39*	6.22
B2: CNTs-NYS	3.00	0.00	50.00	0.47	9.30	104.35	7.15*	8.50	141.55	5.78*	11.15	328.15	7.12*	-19.46
B3: CNTs-FLZ	3.00	0.00	50.00	0.54*	25.58	134.78	6.57	-0.30	121.96	4.03*	-22.50	198.52	6.87*	-22.99
Strategy C														
C1: SLNPs	2.00*	-33.33	0.00	0.31*	-27.91	34.78	3.25*	-50.68	9.80	1.84*	-64.62	36.30	7.04*	-20.36
C2: SLNPs-NYS	3.00	0.00	50.00	0.59*	37.21	156.52	11.29*	71.32	281.42	8.72*	67.69	545.93	6.42*	-27.38
C3: SLNPs-FLZ	3.00	0.00	50.00	0.66*	53.49	186.96	13.39*	103.19	352.36	9.30*	78.85	588.89	6.25*	-29.30

Cont. table 1:

Parameter	Harvest index	% change	% Recov	Crop index	% change	% Recov	Mobilization index	% change	% Recov
Treatment									
Control	0.59	—	—	0.37	—	—	0.042	—	—
Healthy Plant									
CNTs	0.50*	-15.25	—	0.33	-10.81	—	0.041	-2.38	—
CSNPs	1.08*	83.05	—	0.52*	40.54	—	0.062	47.62	—
SLNPs	0.78*	32.20	—	0.44	18.92	—	0.062	47.62	—
Infected Plant with <i>Botrytis fabae</i>									
Infected plant	0.15*	-74.58	—	0.13*	-64.86	—	0.015	-64.29	—
NYS	0.70*	18.64	366.67	0.41	10.81	215.38	0.048	14.29	220.00
FLZ	0.75*	27.12	400.00	0.43	16.22	230.77	0.051	21.43	240.00
Strategy A									
A1: CSNPs	0.31*	-47.46	106.67	0.24*	-35.14	84.62	0.030	-28.57	100.00
A2: CSNPs-NYS	1.16*	96.61	673.33	0.54*	45.95	315.38	0.071	69.05	373.33
A3: CSNPs-FLZ	1.22*	106.78	713.33	0.55*	48.65	323.08	0.078	85.71	420.00
Strategy B									
B1: CNTs	0.19*	-67.80	26.67	0.16*	-56.76	23.08	0.017	-59.52	13.33
B2: CNTs-NYS	0.81*	37.29	440.00	0.45	21.62	246.15	0.063	50.00	320.00
B3: CNTs-FLZ	0.59	0.00	293.33	0.37	0.00	184.62	0.054	28.57	260.00
Strategy C									
C1: SLNPs	0.26*	-55.93	73.33	0.21*	-43.24	61.54	0.029	-30.95	93.33
C2: SLNPs-NYS	1.36*	130.51	806.67	0.58*	56.76	346.15	0.090	114.29	500.00
C3: SLNPs-FLZ	1.49*	152.54	893.33	0.60*	62.16	361.54	0.096	128.57	540.00

Table 2: Effect of foliar application of different nanomaterials treatments either singly or loaded with nystatin and fluconazole on yield developmental parameters of Faba bean plants grown in clay-sandy soil (before and after infection with *Alternaria alternata*). In each case, the % change from the control and the % recovery from the infected plant are also shown. Each value is a mean of five replicates. *The values listed are significantly different from control at $p \geq 0.05$.

Parameter	Plant height (cm plant ⁻¹)	% change	% Recov	Shoot length (cm plant ⁻¹)	% change	% Recov	No. of pods/ plant	% change	% Recov	Pod weight (g pod ⁻¹)	% change	% Recov
Treatment												
Control	61.11	—	—	54.38	—	—	4.00	—	—	1.54	—	—
Healthy Plant												
CNTs	60.32*	-1.29	—	53.21*	-2.15	—	4.00	0.00	—	1.49*	-3.25	—
CSNPs	66.71*	9.16	—	61.31*	12.74	—	5.00*	25.00	—	2.38*	54.55	—
SLNPs	64.12*	4.93	—	59.00*	8.50	—	4.00	0.00	—	1.77*	14.94	—
Infected Plant with <i>Alternaria alternata</i>												
Infected plant	50.46*	-17.43	—	43.52*	-19.97	—	3.00*	-25.00	—	1.02*	-33.77	—
NYS	66.83*	9.36	32.44	58.21*	7.04	33.75	4.00	0.00	33.33	2.05*	33.12	100.98
FLZ	65.62*	7.38	30.04	57.40*	5.55	31.89	4.00	0.00	33.33	2.20*	42.86	115.69
Strategy A												
A1: CSNPs	53.61*	-12.27	6.24	45.28*	-16.73	4.04	3.00*	-25.00	0.00	1.27*	-17.53	24.51
A2: CSNPs-NYS	63.32*	3.62	25.49	55.83*	2.67	28.29	4.00	0.00	33.33	1.79*	16.23	75.49
A3: CSNPs-FLZ	64.21*	5.07	27.25	54.33	-0.09	24.84	5.00*	25.00	66.67	2.29*	48.70	124.51
Strategy B												
B1: CNTs	52.27*	-14.47	3.59	44.34*	-18.46	1.88	3.00*	-25.00	0.00	1.05*	-31.82	2.94
B2: CNTs-NYS	61.73*	1.01	22.33	55.59*	2.23	27.73	4.00	0.00	33.33	1.60	3.90	56.86
B3: CNTs-FLZ	60.15*	-1.57	19.20	54.46	0.15	25.14	5.00*	25.00	66.67	2.20*	42.86	115.69
Strategy C												
C1: SLNPs	55.00*	-10.00	9.00	46.59*	-14.33	7.05	3.00*	-25.00	0.00	1.13*	-26.62	10.78
C2: SLNPs-NYS	69.63*	13.94	37.99	59.76*	9.89	37.32	5.00*	25.00	66.67	2.46*	59.74	141.18
C3: SLNPs-FLZ	70.63*	15.58	39.97	65.28*	20.04	50.00	5.00*	25.00	66.67	2.47*	60.39	142.16

Cont. table 2:

Parameter	No. of seeds/ pod	% change	% Recov	Seed weight (g seed ⁻¹)	% change	% Recov	Crop yield /plant (g plant ⁻¹)	% change	% Recov	Seed yield /plant (g plant ⁻¹)	% change	% Recov	Straw yield /plant (g plant ⁻¹)	% change
Treatment														
Control	3.00	—	—	0.43	—	—	6.59	—	—	5.20	—	—	8.84	—
Healthy Plant														
CNTs	3.00	0.00	—	0.35	-18.60	—	6.37*	-3.34	—	4.07*	-21.73	—	8.14*	-7.92
CSNPs	3.00	0.00	—	0.60*	39.53	—	11.30*	71.47	—	8.97*	72.50	—	8.33*	-5.77
SLNPs	3.00	0.00	—	0.45	4.65	—	7.49*	13.66	—	5.48*	5.38	—	7.04*	-20.36
Infected Plant with <i>Alternaria alternata</i>														
Infected plant	2.00*	-33.33	—	0.24*	-44.19	—	3.06*	-53.57	—	1.40*	-73.08	—	9.76*	10.41
NYS	3.00	0.00	50.00	0.54*	25.58	125.00	8.42*	27.77	175.16	6.52*	25.38	365.71	7.08*	-19.91
FLZ	3.00	0.00	50.00	0.55*	27.91	129.17	8.66*	31.41	183.01	6.62*	27.31	372.86	7.89*	-10.75
Strategy A														
A1: CSNPs	2.00*	-33.33	0.00	0.37	-13.95	54.17	3.76*	-42.94	22.88	2.38*	-54.23	70.00	8.97*	1.47
A2: CSNPs-NYS	3.00	0.00	50.00	0.49	13.95	104.17	7.15*	8.50	133.66	5.56*	6.92	297.14	7.39*	-16.40
A3: CSNPs-FLZ	3.00	0.00	50.00	0.54*	25.58	125.00	10.12*	53.57	230.72	7.67*	47.50	447.86	7.30*	-17.42
Strategy B														
B1: CNTs	2.00*	-33.33	0.00	0.25*	-41.86	4.17	3.08*	-53.26	0.65	1.53*	-70.58	9.29	10.43*	17.99
B2: CNTs-NYS	3.00	0.00	50.00	0.44	2.33	83.33	6.73*	2.12	119.93	5.30*	1.92	278.57	6.94*	-21.49
B3: CNTs-FLZ	3.00	0.00	50.00	0.54*	25.58	125.00	8.81*	33.69	187.91	6.47*	24.42	362.14	6.65*	-24.77
Strategy C														
C1: SLNPs	2.00*	-33.33	0.00	0.33*	-23.26	37.50	3.47*	-47.34	13.40	2.05*	-60.58	46.43	8.11*	-8.26
C2: SLNPs-NYS	3.00	0.00	50.00	0.60*	39.53	150.00	12.18*	84.83	298.04	8.49*	63.27	506.43	7.11*	-19.57
C3: SLNPs-FLZ	3.00	0.00	50.00	0.75*	74.42	212.50	13.21*	100.46	331.70	11.66*	124.23	732.86	6.83*	-22.74

Cont. table 2:

Parameter	Harvest index	% change	% Recov	Crop index	% change	% Recov	Mobilization index	% change	% Recov
Treatment									
Control	0.59	—	—	0.37	—	—	0.042	—	—
Healthy Plant									
CNTs	0.50*	-15.25	—	0.33	-10.81	—	0.041	-2.38	—
CSNPs	1.08*	83.05	—	0.52*	40.54	—	0.062	47.62	—
SLNPs	0.78*	32.20	—	0.44	18.92	—	0.062	47.62	—
Infected Plant with <i>Alternaria alternata</i>									
Infected plant	0.14*	-76.27	—	0.13*	-64.86	—	0.013	-69.05	—
NYS	0.92*	55.93	557.14	0.48*	29.73	269.23	0.068	61.90	423.08
FLZ	0.84*	42.37	500.00	0.46*	24.32	253.85	0.058	38.10	346.15
Strategy A									
A1: CSNPs	0.27*	-54.24	92.86	0.21*	-43.24	61.54	0.023	-45.24	76.92
A2: CSNPs-NYS	0.75*	27.12	435.71	0.43	16.22	230.77	0.058	38.10	346.15
A3: CSNPs-FLZ	1.05*	77.97	650.00	0.51*	37.84	292.31	0.070	66.67	438.46
Strategy B									
B1: CNTs	0.15*	-74.58	7.14	0.13*	-64.86	0.00	0.012	-71.43	-7.69
B2: CNTs-NYS	0.76*	28.81	442.86	0.43	16.22	230.77	0.062	47.62	376.92
B3: CNTs-FLZ	0.97*	64.41	592.86	0.49*	32.43	276.92	0.074	76.19	469.23
Strategy C									
C1: SLNPs	0.25*	-57.63	78.57	0.20*	-45.95	53.85	0.025	-40.48	92.31
C2: SLNPs-NYS	1.19*	101.69	750.00	0.54*	45.95	315.38	0.077	83.33	492.31
C3: SLNPs-FLZ	1.71*	189.83	1121.43	0.63*	70.27	384.62	0.092	119.05	607.69

Biosafety of yielded *Vicia faba* seeds

After confirming the presence of tested nanomaterials (CSNPs, CNTs and SLNPs) singly or loaded with nystatin and fluconazole inside plant tissue, in particular phloem tissue (sieve tubes), it was necessary to figure out the final fate of these nanomaterials. To this end, TEM analysis of representative yielded seeds from *Vicia faba* plants that had undergone various treatments was carried out.

Careful examination of figure 7 revealed the following main points:

- Yielded seeds produced from *Vicia faba* plants treated with different nanomaterials loaded with nystatin and fluconazole antifungal antibiotics exhibited complete absence of nanomaterials inside their tissues.
- When compared with control yielded *Vicia faba* seeds tissues, no change in seed tissues of the variously nanomaterials-treated plants as nano-drug delivery strategies which clearly indicates the complete absence of the different nanomaterials inside yielded *Vicia faba* seeds tissues.

In conclusion, comparing the three new and novel nano-drug delivery strategies under investigation, in the present study from the percentage important and recovery from illness of broad bean plants diseased with chocolate spot or leaf spot, the following sequence of treatments (nano-drug delivery strategy C > nano-drug delivery strategy A > nano-drug delivery strategy B) was, in general, displayed with respect to recovery from chocolate spot disease caused by *Botrytis fabae* fungal pathogen. On the other hand, except for the results obtained for mobilization index of broad bean yield, the following sequence of treatments (nano-drug delivery strategy C > nano-drug delivery strategy A > nano-drug delivery strategy B) was, in general, displayed with respect to all growth and developmental yield parameters of the percentage improvement and recovered plants from illness with leaf spot disease caused by *Alternaria alternata* fungal pathogens.

Discussion

b. Uptake and translocation

It is indicated from TEM images 1–6 the complete absence of nanomaterials either singly or loaded with nystatin and fluconazole of healthy, diseased and recovered broad bean plants inside epidermal cells, cuticle cells, spongy and palisade leaf tissues but they were existed in vascular system of broad bean

leaves treated with the three different nanomaterials either singly or loaded with nystatin and fluconazole. Careful examination of images 1–6, in the present study, showed that foliar application of CSNPs, CNTs or SLNPs either singly or loaded with nystatin and fluconazole of broad bean plants and the translocation of nanomaterials was achieved by phloem tissues, in particular, sieve tubes of broad bean plants. These novel and innovative results indicate clearly the ability of CSNPs, CNTs or SLNPs to penetrate leaf phloem tissues of broad bean plants then move quickly downwards towards stem vascular tissues and root vascular tissues. It is worth to mention that the observed results concerning the uptake and translocation of nanomaterials by broad bean plants through the entire period of the experiment might lead to conclude that the main pathway for uptake and translocation of nanomaterials either singly or loaded with nystatin and fluconazole was via the cells of the phloem sieve tubes of leaves, stems and finally downwards to roots.

In connection with the present results [18, 19] reported that the diffusion of foliarly sprayed engineered nanomaterials when cross the cell walls pores in the space between the cell wall and plasma membrane is subject to capillary forces or osmotic pressure. Through this route, nanoparticles may enter epidermal and cortical cells till reach the endodermis. However, aggregates accumulate in the endodermis due to the presence of waxy Casparian strip. engineered nanomaterials in apoplastic route must integrate into the symplastic route to penetrate into vascular system to ensure effective translocation to the shoot system [20]. The symplastic route is a highly regulated path for transporting engineered nanomaterials. Upregulation of water channel genes and enhanced aquaporin proteins expression supported the passive uptake mechanisms [21].

c. Changes in yield growth and developmental parameters

In the present study, results recorded in Tables 1 and 2 indicated that cell growth and developmental yield parameters of healthy, diseased and recovered *Vicia faba* plants treated with *Botrytis fabae* and *Alternaria alternata* and nanoparticles either singly or loaded with antibiotics, as new and novel nano-drug delivery strategies were significantly and variously increased. Meanwhile, harvest index, crop index and mobilization index were variously changed in healthy, infected and recovered *Vicia faba* plants when compared with control. It is of interest in this work to mention that nano-drug delivery strategies A, B and C, as new and novel strategies for controlling chocolate spot disease and leaf spot disease of *Vicia faba* infected with *Botrytis fabae* and *Alternaria alternata*, showed significant recovery from diseases in case of crop index parameter of recovered *Vicia faba* plants by a ratio of the following: : 361.5% for SLNPs + FLZ, 346.1% for SLNPs + NYS, 323.1% for CSNPs + FLZ, 315.4% for CSNPs + NYS, 246.1% for CNTs + NYS and 184.6% for CNTs + FLZ in case of *Botrytis fabae* and was of: 384.6% for SLNPs + FLZ, 315.3% for SLNPs + NYS, 292.3% for CSNPs + FLZ, 276.9% for CNTs + FLZ, 230.7% for CSNPs + NYS and 230.7% for CNTs + NYS in case of *Alternaria alternata*.

The increased *Vicia faba* crop index of yielded seeds upon treatment of infected plants with either *Botrytis fabae* or *Alternaria alternata* fungal pathogens with either antifungal antibiotics or nano-drug delivery strategies A, B and C proved the significance of nano-drug delivery strategies as new and novel strategies for management and controlling of plant diseases. A new and novel trials for using nanomaterials either singly or loaded with nystatin and fluconazole antifungal antibiotics to evaluate their antifungal effects against *Botrytis fabae* and *Alternaria alternata* the causal agents of chocolate spot and leaf spot diseases of faba bean.

It was reported that the tested nanoparticles with antifungal antibiotics showed significant effects against *Botrytis fabae* and *Alternaria alternata* fungal pathogens in relation to control [7, 22]. The present results agree with the findings of [23] who reported that silver nanoparticles have been used to prevent some plant pathogens. Also, [23] suggested that direct contact of nanoparticles with fungal spores or germ tubes is critical in inhibiting disease development. It could be concluded that nanomaterials loaded with antifungal antibiotics, as new nano-drug delivery system, in the present study, could be used effectively in control of chocolate spot and leaf spot of faba bean and prevention of deleterious infections. These nanomaterial types may be less toxic to human and environment. The results of this study focus on extended applicability of nanomaterials either singly or loaded with antifungal antibiotics, as new and novel nano-drug delivery systems for management and controlling of *Botrytis fabae* and *Alternaria alternata* [22].

d. Biosafety of the yielded seeds

Nanomaterials can be transferred from plants to ecosystem and human by harvested organs causing toxicity to organisms and affecting the abundance and biodiversity of these ecosystems. It is worth noting from the present results (see Fig. 7) that yielded *Vicia faba* seeds were completely free from any applied nanomaterials implying that these nanomaterials were not stored inside yielded seeds tissues (see Fig. 7), as aggregation, translocation and distribution of engineered nanomaterials play an important role in their fate once they entered into the plant tissues and their fate within the tissues was different [24, 25]. In connection with these studies, it was reported the possibility of nanomaterials to transfer through the food chain with poorly formed life cycle hazards to health and safety and with probable adverse effects on the environment [26–28]. Thus, it should be cautious to compare the antifungal activity of the various types of nano-systems. Nevertheless, the comparison of antifungal effect of the nanoparticles with a possible control antifungal approved for field application is suggested.

Conclusion

When considering the formulation design for antimicrobial therapy, it is important to develop the nanocarriers that can stabilize the active ingredients and improve delivery into the infection site. Increasing attention has been paid to nano agriculture as an approach for applying microbial targeting or delivery. The use of antibiotic-loaded nanoparticles is considered a valid strategy for microbial targeting because of its numerous advantages over conventional formulations, including improved stability, controlled antibiotic release, targeted capability, and increased bioavailability. Some issues, such as solubility and drug resistance can also be resolved by nanocarriers, targeting pathogens. The present investigation clearly showed application of three nano-drug delivery strategies for controlling two fungal pathogens: *Botrytis fabae* and *Alternaria alternata* which cause major disease chocolate spot and minor disease leaf spot of *Vicia faba* plants. Solid lipid nanoparticles, chitosan nanoparticles and carbon nanotubes loaded with nystatin or fluconazole nanocarriers applied to control the two fungal diseases infect *Vicia faba* plants. The net results indicate the possibility of these formulations to protect plants from the diseases with a sequence of solid lipid nanoparticles > chitosan nanoparticles > carbon nanotubes.

Declarations

- **Ethics approval and consent to participate:** Not applicable
- **Consent for publication:** All authors read and agreed to submit the manuscript.
- **Availability of data and materials:** All data generated or analyzed during this study are included in this article.
- **Competing interests:** The authors have no conflicts of interest to declare that are relevant to the content of this article.
- **Funding:** Not applicable
- **Authors' contribution:**

Conceptualization, M.N.A.H., and H.M.M.A.-A.; methodology, S.H.H. and H.M.M.A.-A.; software, S.H.H. and H.M.M.A.-A.; validation, M.M.E.; formal analysis, S.H.H. and H.M.M.A.-A.; investigation, S.H.H.; resources, M.N.A.H., and H.M.M.A.-A.; data curation, S.H.H. and H.M.M.A.-A.; writing—original draft preparation, S.H.H., H.M.M.A.-A. and M.N.A.H.; writing—review and editing, H.M.M.A.-A. and M.N.A.H.; visualization, M.M.E., M.N.A.H. and H.M.M.A.-A.; supervision, M.M.E., M.N.A.H. and H.M.M.A.-A.; project administration, M.M.E., M.N.A.H. and H.M.M.A.-A.

References

1. Ramezani, M., Ramezani, F. & Gerami, M. Nanoparticles in pest incidences and plant disease control. *Nanotechnology for Agriculture: Crop Production and Protection*. 233-272 (2019).
2. Iavioli, I., Leso, V., Beezhold, D.H. & Shvedova, A.A. Nanotechnology in agriculture: Opportunities, toxicological implications, and occupational risks. *Toxicol. Appl. Pharmacol.* **329**, 96-111 (2017).
3. Adetunji, C., Oloke, J., Kumar, A., Swaranjit, S. & Akpor, B. Synergetic effect of rhamnolipid from *Pseudomonas aeruginosa* C1501 and phytotoxic metabolite from *Lasiodiplodia pseudotheobromae* C1136 on *Amaranthus hybridus* L. and *Echinochloa crus-galli* weeds. *Environ. Sci. Pollut. Res.* **24**, 13700-13709 (2017).
4. Rosenthal, J.A., Chen, L., Baker, J.L., Putnam, D. & DeLisa, M.P. Pathogen-like particles: biomimetic vaccine carriers engineered at the nanoscale. *Curr. Opin. Biotechnol.* **28**, 51-58 (2014).
5. Voltan, A.R., Quindos, G., Alarcón, K.P.M., Fusco-Almeida, A.M., Mendes-Giannini, M.J.S. & Chorilli, M. Fungal diseases: could nanostructured drug delivery systems be a novel paradigm for therapy?. *Int. J. Nanomedicine* **11**, (2016).
6. Mahmoud, N.M. Studies on chocolate spot disease of broad bean and loss occurrence. *Ph.D. Thesis, Fac. Agric., Minufiya University, Egypt*. p. 133 (1996).
7. Ahmed, A.I.S. Chitosan and silver nanoparticles as control agents of some Faba bean spot diseases. *J. plant pathol. microbiol.* **8**, (2017).
8. WHO. Options for Action. Geneva: WHO Publication (2012).
9. Sardi, J.C.O., Pitangui, N.S., Rodríguez-A.G., Taylor, M.L., Fusco-Almeida, A.M. & Mendes-Giannini, M.J.S. Highlights in pathogenic fungal biofilms. *Rev. Iberoam Micol* **31**, 22-29 (2014).
10. Pelgrift, R.Y. & Friedman, A.J. Nanotechnology as a therapeutic tool to combat microbial resistance. *Adv. Drug Deliv. Rev.* **65**, 1803-1815 (2013).
11. Abdel-Aziz, H.M.M., Hasaneen, M.N.A. & Omar, A.M. Effect of foliar application of nano chitosan NPK fertilizer on the chemical composition of wheat grains. *Egypt. J. Bot.* **58**, 87-95 (2018).
12. Harrison, J.G. Overwintering of *Botrytis fabae*. *Trans. Br. Mycol. Soc.* **72**, 389-394 (1979).
13. Hanounik, S.B. & Hawtin, G.C. Screening for resistance to chocolate spot caused by *Botrytis fabae*. Faba Bean Improvement (Hawtin, G.C. and Webb, C., Eds.). Martinus Nijhoff Publishers, The Hague, The Netherlands. 243-250 pp (1982).
14. Singh, D., Sangle, U.R., Kumar, B., Tripathi, H.S., Singh, K.P. & Gupta, A.K. Integrated disease management of Faba bean (*Vicia faba* L.). Faba Bean: A Potential Leguminous Crop of India, 279-301 (2012).
15. Narain, U. & Saxena, H. K. Leaf spot and head rot of sunflower caused by *Alternaria alternata*. *Indian Phytopathol.* **34**, 128 (1981).
16. Nishimura, S. & Kohmoto, K. Host specific toxins and chemical structures from *Alternaria* species. *Annu. Rev. Phytopathol.* **2**, 87-116 (1983).
17. Lagopodi, A.L. & Thanassouloupoulos, C.C. Effect of a leaf spot disease caused by *Alternaria alternata* on yield of sunflower in Greece. *Plant Dis.* **82**, 41-44 (1998).
18. Lin, S., Reppert, J., Hu, Q., Hunson, J.S., Reid, M.L., Ratnikova, T., Rap, A.M., Luo, H. & Ke, P.C. Uptake, translocation and transmission of carbon nanomaterials in rice plants. *Small* **5**, 1128-1132 (2009).
19. Hasaneen, M.N.A., Abdel-Aziz, H.M.M. & Omer, A.M. Effect of foliar application of engineered nanomaterials: carbon nanotubes NPK and chitosan nanoparticles NPK fertilizer on the growth of French bean plant. *Biochemistry Biotechnology Research* **4**, 68-76 (2016).
20. Larue, C., Pinault, M. & Czarny, B. Quantitative evaluation of multiwalled carbon nanotube uptake in wheat and rape seed. *J. Hazard. Mater.* **228**, 155-163 (2012).
21. Khodakovskaya, M., de Silva, K., Dervishi, E., Biris, A.S. & Villagarcia, H. Carbon nanotubes induce growth enhancement of tobacco cells. *ACS Nano* **6**, 2128-2135 (2012).
22. Helal, S.H., Hasaneen, M.N.A., El-Zayat, M.M. & Abdel-Aziz, H.M.M. *In vitro* efficacy of antifungal activities either singly or loaded on nanomaterials, as new and novel drug delivery systems on the growth of *Alternaria alternata* and *Botrytis fabae* fungal pathogens. *Mans J Biol.* **41**, (2022).
23. Kasproicz, M.J., Koziol, M. & Gorczyca, A. The effect of silver nanoparticles on phytopathogenic spores of *Fusarium culmorum*. *Can. J. Microbiol.* **56**, 247-253 (2010).

24. Young, K.J., Byung, H.K. & Geunhwa, Jung. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. *Plant Dis.*1037-1043 (2009).

25. Murashov, V. & Howard, J. Biosafety, Occupational Health and Nanotechnology. *Appl. Biosaf.* **12**, 158-167 (2007).

26. Marchiol, L., Mattiello, A., Pošćic, F., Fellet, G., Zavalloni, C., Carlino, E. & Musetti, R. Changes in physiological and agronomical parameters of barley (*Hordeum vulgare*) exposed to cerium and titanium dioxide nanoparticles. *Int. J. Environ. Res. Public Health* **13**, 1-18 (2016).

27. Rao, V. Systems approach to biosafety and risk assessment of engineered nanomaterials. *Appl. Biosaf.* **19**, 11-19 (2014).

28. Deng, Y., White, J.C. & Xing, B. Interactions between engineered nanomaterials and agricultural crops: implications for food safety. *J. Zhejiang Univ. Sci.* **15**, 552-572 (2014).

Figures



Figure 1

Effect of foliar application of different treatments on *Vicia faba* plants infected with *Botrytis fabae*during flowering stage.



Figure 2

Effect of foliar application of different treatments on *Vicia faba* plants infected with *Botrytis fabae* during flowering stage.



Figure 3

Effect of foliar application of different treatments on *Vicia faba* plants infected with *Alternaria alternata* during flowering stage.



Figure 4

Effect of foliar application of different treatments on *Vicia faba* plants infected with *Alternaria alternata* during flowering stage.

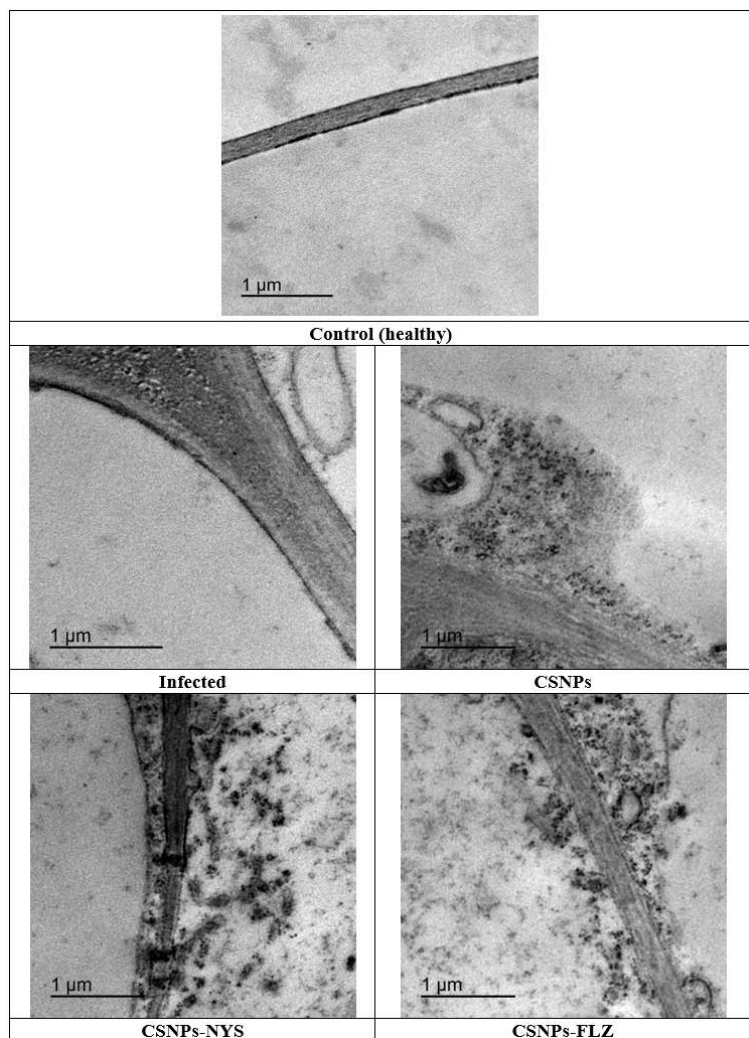


Figure 5

Fig. 4: TEM micrograph of phloem tissue of healthy, infected with *Botrytis fabae* and recovered 46-day-old *Vicia faba* leaves treated with chitosan nanoparticles (CSNPs) either singly or loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics.

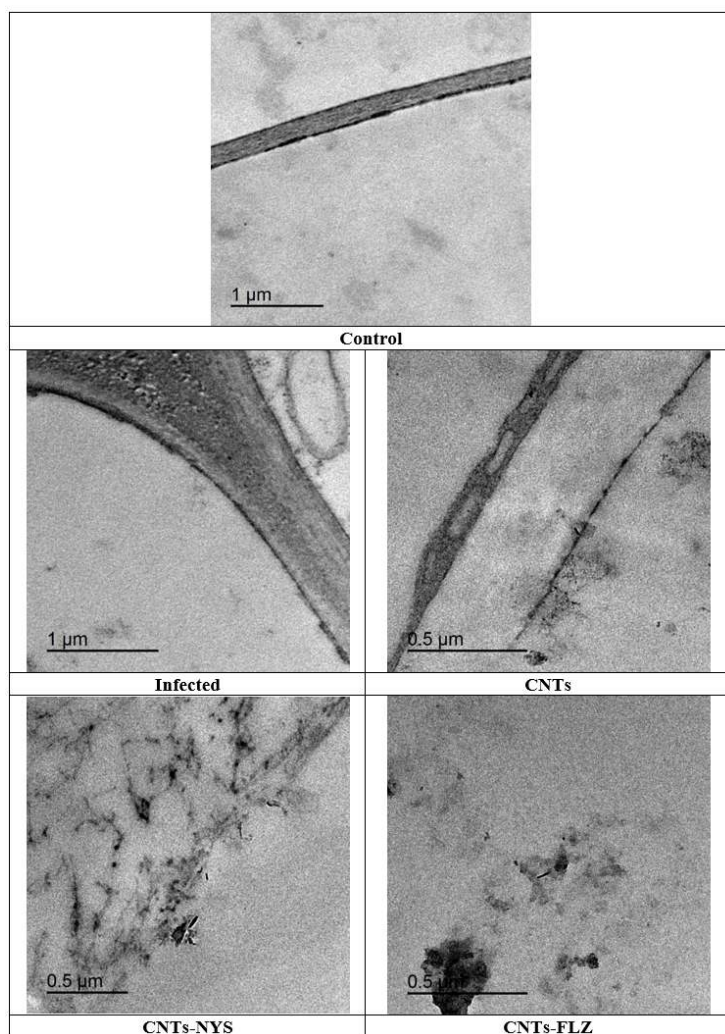


Figure 6

Fig. 5: TEM micrograph of phloem tissue of healthy, infected with *Botrytis fabae* and recovered 46-day-old *Vicia faba* leaves treated with carbon nanotubes (CNTs) either singly or loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics

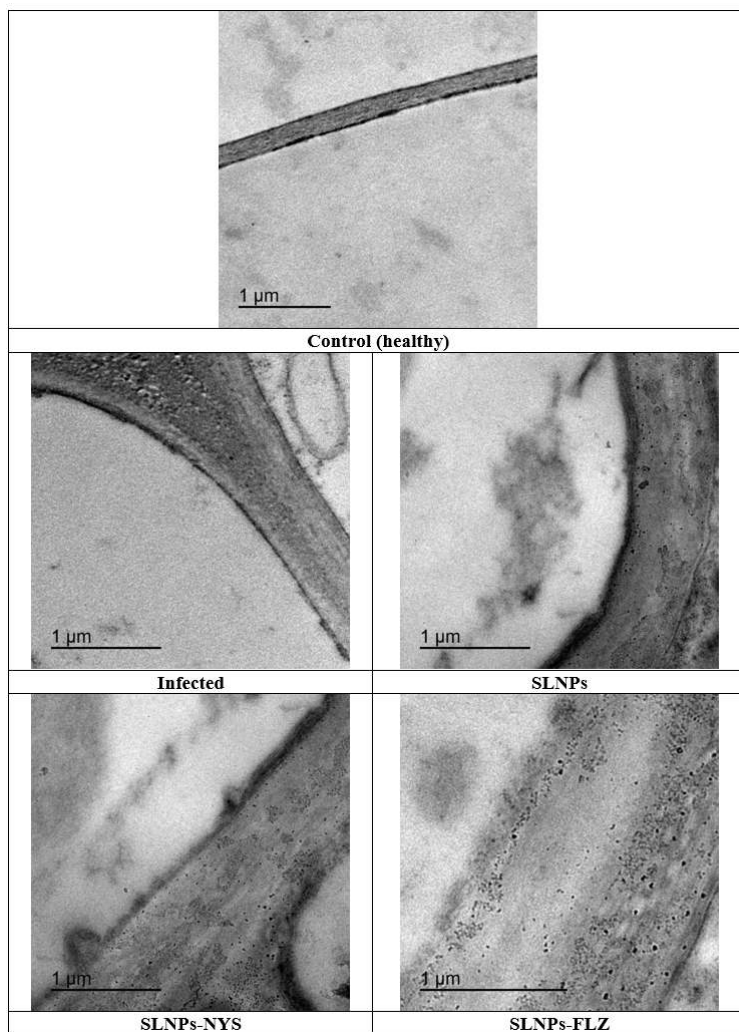


Figure 7

Fig. 6: TEM micrograph of phloem tissue of healthy, infected with *Botrytis fabae* and recovered 46-day-old *Vicia faba* leaves treated with solid lipid nanoparticles (SLNPs) either singly or loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics

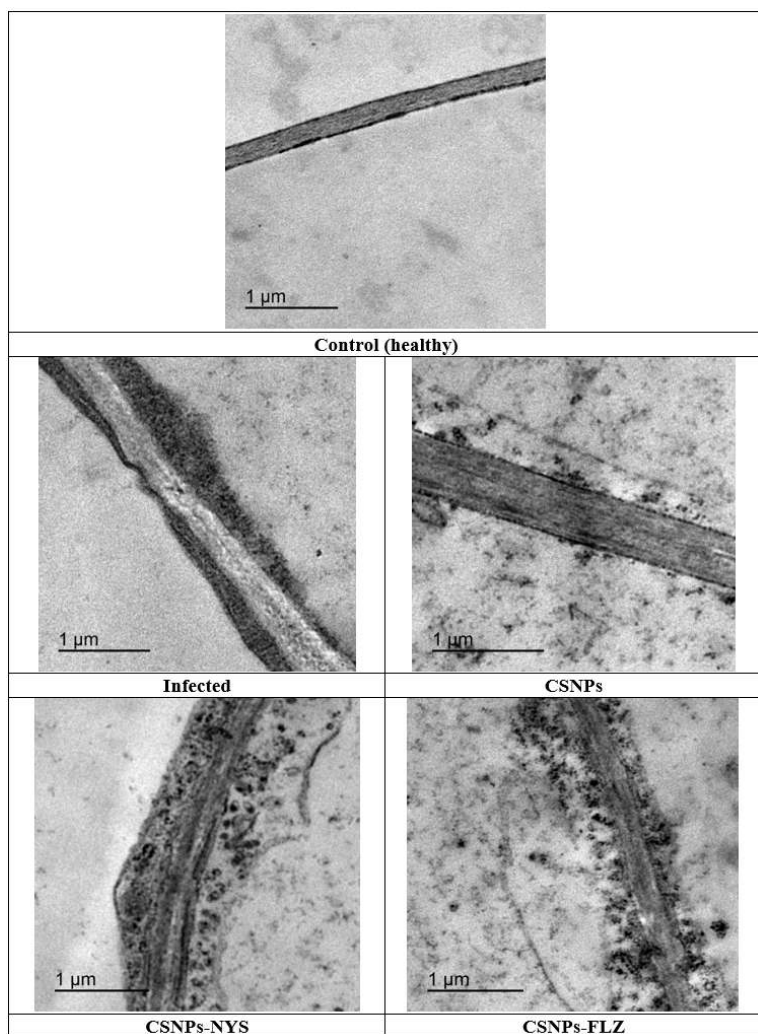


Figure 8

Fig. 7: TEM micrograph of phloem tissue of healthy, infected with *Alternaria alternata* and recovered 46-day-old *Vicia faba* leaves treated with chitosan nanoparticles (CSNPs) either singly or loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics

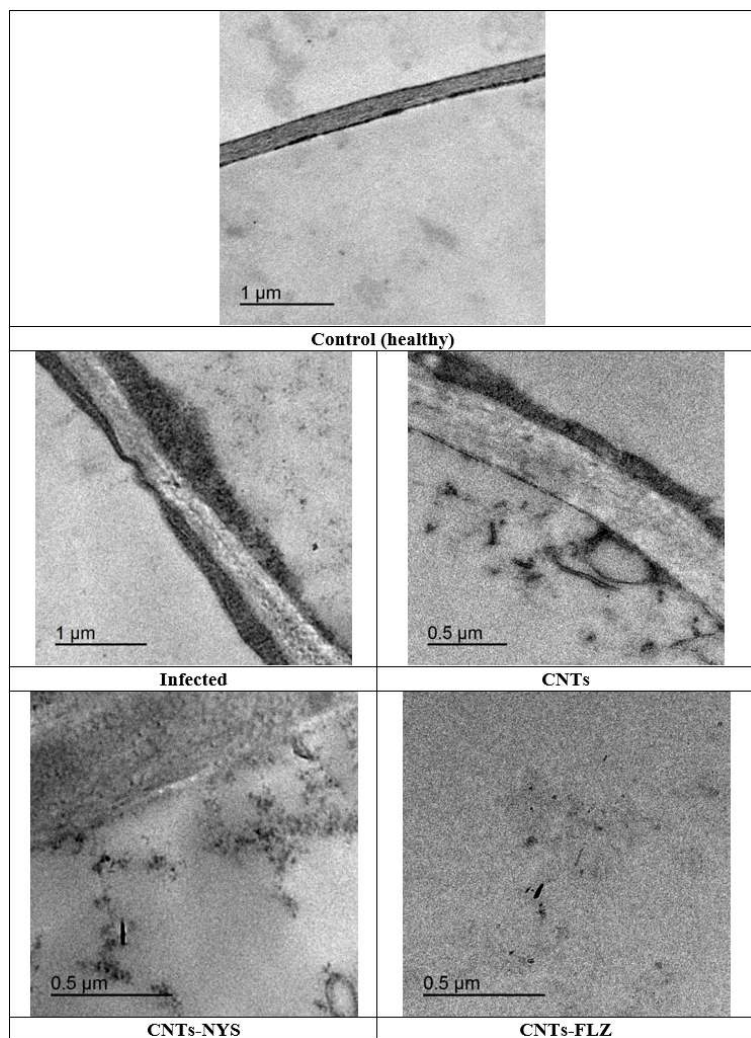


Figure 9

Fig. 8: TEM micrograph of phloem tissue of healthy, infected with *Alternaria alternata* and recovered 46-day-old *Vicia faba* leaves treated with carbon nanotubes (CNTs) either singly or loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics

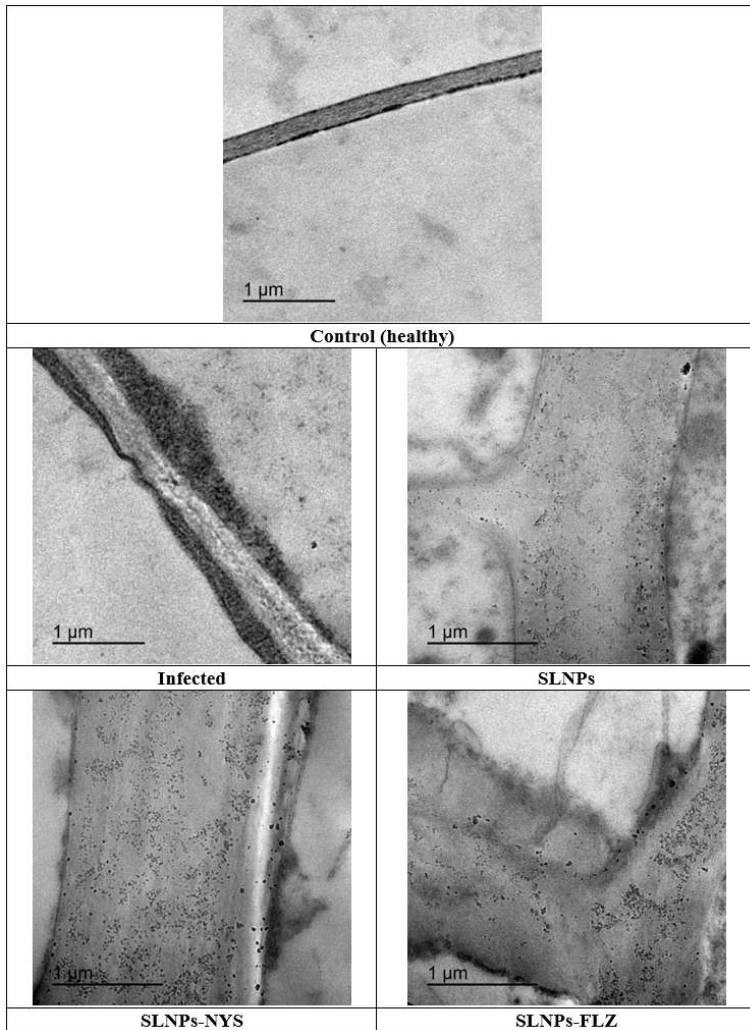


Figure 10

Fig. 9: TEM micrograph of phloem tissue of healthy, infected with *Alternaria alternata* and recovered 46-day-old *Vicia faba* leaves treated with solid lipid nanoparticles (SLNPs) either singly or loaded with nystatin (NYS) and fluconazole (FLZ) antifungal antibiotics.\

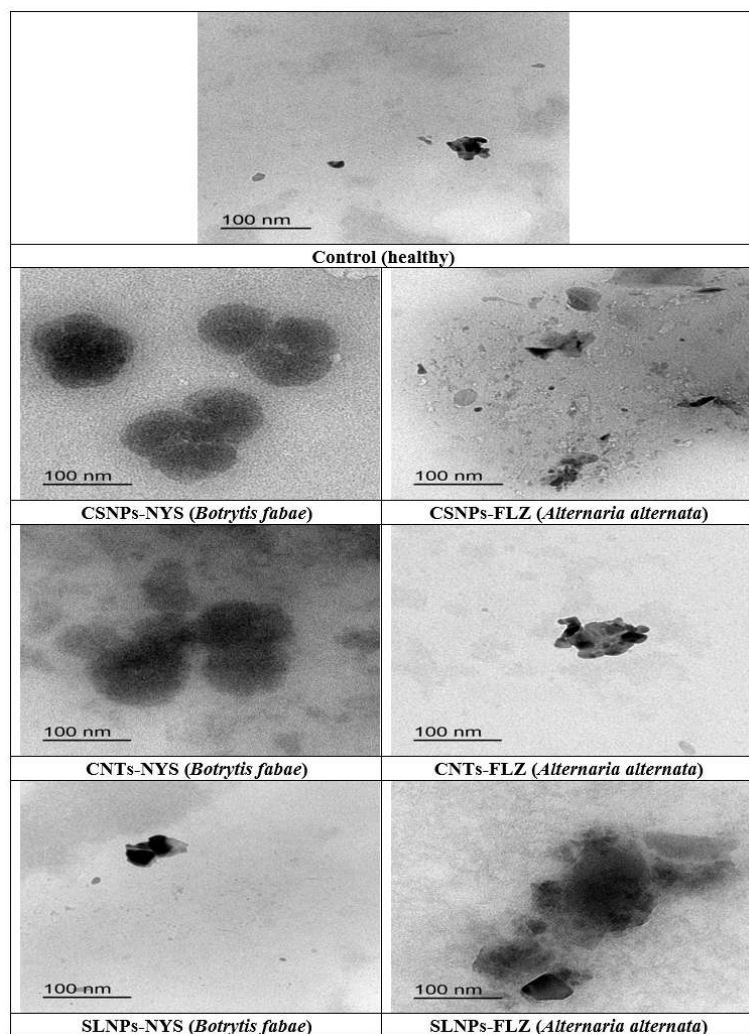


Figure 11

Fig. 7: TEM micrographs of representative yielded seeds from *Vicia faba* plants treated with different tested nanomaterials loaded with nystatin and fluconazole as nano-drug delivery systems.